Biochimica et Biophysica Acta 1787 (2009) 1499–1504

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Filling the "green gap" of the major light-harvesting chlorophyll *a*/*b* complex by covalent attachment of Rhodamine Red

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ARTICLE INFO

Article history:
Received 8 May 2009
Received in revised form 10 July 2009
Accepted 13 July 2009
Available online 18 July 2009

Keywords:
FRET (Förster resonance energy transfer)
LHCII
Maleimide dye
Photosynthesis
Site-specific labeling
Solar spectrum

ABSTRACT

The major light-harvesting chlorophyll *a*/*b* complex (LHCII) greatly enhances the efficiency of photosynthesis in green plants. Recombinant LHCII can be assembled in vitro from its denatured, bacterially expressed apoprotein and plant pigments. This makes it an interesting candidate for biomimetic light-harvesting in photovoltaic applications. Due to its almost 20 pigments bound per apoprotein, LHCII absorbs efficiently in the blue and red spectral domains of visible light but less efficiently in the green domain, the so-called "green gap" in its absorption spectrum. Here we present a hybrid complex of recombinant LHCII with organic dyes that add to LHCII absorption in the green spectral region. One or three Rhodamine Red dye molecules were site-specifically attached to cysteine side chains in the apoprotein and did not interfere with LHCII assembly, function and stability. The dyes transferred their excitation energy virtually completely to the chlorophylls in LHCII, partially filling in the green gap. Thus, organic dyes can be used to increase the absorption cross section and, thus, the light-harvesting efficiency of recombinant LHCII.

1. Introduction

The photosynthetic apparatus in plants is the result of a 3-billion year evolution process to optimize its efficiency in converting solar energy primarily into an electrochemical potential and then into other forms of energy. Therefore, many attempts have been made to adopt structural components of the natural solar energy converter to improve technical applications such as photovoltaic devices [1]. Some of these biomimetic approaches included biological components such as photosystems attached to a metal or semiconductor surface to produce a photovoltaic cell [2,3]. Others adopted structural principles of the natural model; one example of this approach is the Grätzel solar cell using sensitizer dyes, mimicking photosynthetic reaction centers, for light-driven separation of electrons and positive charges [4].

All natural photosynthetic systems, whether in plants or bacteria, contain light-harvesting components. These increase the efficiency of photosynthesis by collecting light energy and conducting the excitation energy to the photosynthetic reaction center where the actual charge separation takes place [5]. Photovoltaic devices, too, have made use of this principle [6]. Again, one possibility for adding a light-harvesting function to a photovoltaic device is to include the corresponding biological structures, the light-harvesting complexes from photosynthesizing organisms. Several natural light harvesters have been proposed for this purpose. These include self-organized aggregates of bacteriochlorophyll *c* (BChl *c*), mimicking the chlorosome structure in green sulfur bacteria [7], the ring-shaped light-harvesting complexes LH1 and LH2 from purple bacteria [8], peridinin-chlorophyll proteins (PCP) from dinoflagellates [9], and the major light-harvesting complex LHCII from green plants [10,11]. The latter is a particularly promising candidate since it has a high density of pigments; the concentration of chlorophylls (Chl) in LHCII crystals amounts to 0.3 M [12] which cannot be achieved in organic solvents without massive concentration quenching of excited Chl [13]. The Chl density in LHCII is only topped by that in chlorosomes, in which BChl *c* self-organizes in the absence of a protein scaffold [14,15]. On the other hand, the protein moiety in LHCII is advantageous for integrating the complex into an artificial device since the recombinant version of LHCII can be made with a large variety of binding anchors such as histidine or biotin tags and sulfhydryls for attaching it to other components [16,11]. An extra plus of LHCII may be the possibility to switch it back and forth between a light-harvesting and energy dissipating state by a conformational change [17]; this possible functional switch, however, is still under debate [18].

One disadvantage of LHCII for its potential use as a light-harvesting component is its incomplete absorption of the solar light spectrum. LHCII absorbs well in the blue and red spectral domains but in the green gap, between 500 and 600 nm, its absorption is rather low. An attempt has been described to fill the green gap in the photosynthesis action spectra of isolated chloroplasts by adding Rhodamine-labeled lipids to the thylakoid membrane [19]; the Rhodamine molecules stochastically distributed in the membrane would then transfer their excitation energy to a Chl provided they were close enough to one of the photosystems. In this work we demonstrate that the green gap can at least partially be filled by attaching one ore more Rhodamine dyes

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doi:10.1016/j.bbabio.2009.07.003

directly to the apoprotein of LHCII. A similar approach has been reported to fill the green gap in chlorosome-like Zn-chlorin assemblies by attaching a naphthalene bisimide dye to each Zn-chlorin molecule [20].

2. Materials and methods

2.1. LHCII mutants

For reconstitution of LHCII, two bacterially expressed apoprotein derivatives of *Lhcb1*2 (AB80)* from *Pisum sativum* [21] were used. The first contains a single cysteine at position 3 (S3Ch), the second three cysteines at positions 3, 106, 160 (3xCh). In both mutants, the native Cys79 is exchanged with serine and a hexahistidyl (His_6) tag is added to the C-terminus. The construction of a plasmid coding for S3C (as S3Ch but without the His_6 tag) was described in Wolf-Klein et al. [10]. The His_6 tag was added as specified elsewhere for the construct C2.4 h [16]. The plasmid coding for 3xCh was constructed by ligating the appropriate EcoRI/XhoI restriction fragments of plasmids coding for the double mutant S3C/S106C and the single mutant S160Ch [22].

2.2. Preparation and labeling of LHCII apoprotein

The proteins were expressed in *Escherichia coli* as described earlier [23]. For labeling the proteins were dissolved in 0.5% (w/v) lithium dodecyl sulfate, 20 mM sodium phosphate (pH 7) to a final protein concentration of 1 mg/ml and denatured by heat treatment (2 min, 100 °C). The cysteines were reduced by adding 2 mM tris-(2-cyanoethyl)phosphine and incubation for 2 h at 37 °C. Rhodamine Red® C2 maleimide (Invitrogen, Germany) was dissolved in dimethyl sulfoxide (DMSO) and added to the solution in a 15-fold molar excess over cysteine (the final DMSO concentration never exceeded 4% (v/v)). Afterwards the samples were incubated at 37 °C over night. The proteins were precipitated by adding 0.1 volume of 100 mM acetic acid and 2.3 volumes of acetone and incubation at −20 °C for at least 3 h. The protein was collected by centrifugation (22 000 ×*g* for 15 min at 4 °C), washed several times with 70% ethanol and once with 100% ethanol and dried at room temperature for 5 min.

2.3. Preparation of recombinant LHCII trimers

Total pigment extract, chlorophylls *a* and *b* (Chl *a*; Chl *b*) and carotenoids were isolated from pea thylakoids [24]. Unlabeled and labeled LHCII apoproteins were reconstituted to monomers by the detergent exchange method [25] and trimerization was carried out by affinity chromatography [26]. For separating monomers and trimers and for removing unbound pigments and unfolded protein, the reconstitution solution was ultracentrifuged through a sucrose density gradient as described earlier [27].

2.4. Absorption measurements

Absorption spectra were measured at room temperature using a V550 UV–Vis spectrophotometer (Jasco, Germany). The concentration of LHCII was determined by measuring the absorption at 670 nm ($\varepsilon = 546{,}000\ M^{-1}\ cm^{-1}$; [28]). Difference spectra between labeled and unlabeled samples were computed and the resulting dye spectra with their maximum at 573 nm were used to calculate dye concentrations ($\varepsilon = 119{,}000\ M^{-1}\ cm^{-1}$; according to the manufacturer) and, thus, labeling stoichiometries, assuming that the extinction coefficient remains unchanged upon protein binding.

2.5. Fluorescence measurements

Fluorescence measurements (emission spectra: $\lambda_{ex} = 470$ nm and 550 nm, excitation spectra: $\lambda_{em} = 680$ nm) were recorded with a Fluoromax-2 spectrometer (Jobin Yvon, Germany). LHCII samples to be measured were diluted to 37 nM ($A_{670} = 0.02$), free dye samples had the same concentration as dye in labeled samples. Fluorescence emission spectra were corrected for the wavelength-dependent sensitivity of the fluorimeter.

For energy transfer calculations, emission spectra of LHCII and dye were deconvoluted and fitted spectra, representing only the dye or LHCII contribution to the measured spectra, were integrated with the rectangle rule.

2.6. CD measurements

CD spectra were measured with a J-810-S spectropolarimeter (Jasco, Germany) equipped with a temperature regulating unit. Correct folding and oligomerization was verified by steady state measurements [23]. For stability measurements the signal at 492 nm was monitored over a temperature range from 20 °C to 80 °C (data pitch 0.2 °C, temperature slope 1 °C/min). The data were fitted to a sigmoidal function with Table Curve 2D 4.0 (SPPS Inc, USA) and the quality of the fits was assessed using residual R^2 criteria and plots of residuals. The temperatures at the inflection points of the fitted curves were taken as a measure of complex stability.

2.7. Pigment analysis

Pigments of LHCII samples were extracted with sec-butanol similar to [29]. LHCII samples were mixed with 2/3 vol of sec-butanol and 1/3 vol of 5 M NaCl, mixed thoroughly and centrifuged for 1 min at 22000 ×*g*. The butanol phase containing the pigments was diluted 2-fold with 70% (v/v) acetone and analyzed via HPLC (RP-18 HPLC column, Chromolith SpeedROD, Merck, Germany). The pigments were eluted by a gradient of 70%–100% (v/v) acetone at a flow rate of 1.5 ml/min. Quantitation was performed as in [23] on a pigment per 2 lutein level.

2.8. Determination of fluorescence quantum yield and Förster distances

The fluorescence quantum yield (φ_F) of Rhodamine Red was measured at room temperature in 0.1% (w/v) β-dodecylmaltoside using water-soluble perylene maleimide in water as a reference [30] and determined as 0.5; φ_F of LHCII was determined likewise as 0.2 with Fluorescein as a reference [31].

The Förster distance (R_0) of Rhodamine Red to Chl *a* and Chl *b* was calculated with the following equation [32]

$$R_0 = 0.211\left[\kappa^2 * \eta^{-4} * \varphi_F * J(\lambda)\right]^{1/6} \quad \text{Å}. \tag{1}$$

Here κ^2 is the orientation factor, η the refractive index of the solvent, φ_F the fluorescence quantum yield of the donor (Rhodamine Red) and $J(\lambda)$ the overlap integral between donor emission and acceptor absorption spectra. As a first approximation $\kappa^2 = 2/3$ (for randomly oriented dipole moments) and $\eta = 1.54$ (refractive index for protein environment [33]) were used. The overlap integral was calculated with [32]

$$J(\lambda) = \left[\int F_D(\lambda) * \varepsilon_A * \lambda^4 d\lambda\right] / \left[\int F_D(\lambda) d\lambda\right] \quad \text{mol} * l^{-1} * \text{cm}^{-1} * \text{nm}^4. \tag{2}$$

F_D is the fluorescence intensity of the donor (Rhodamine Red), ε_A the molar extinction coefficient of the acceptor (Chl) and λ the wavelength.

2.9. Distance estimates based on the LHCII crystal structure

Distances inside the LHCII complex were calculated center to center with Swiss-PdbViewer 4.0 [34] using the pdb entry 2BHW [12].

The distance between the S atom of cysteine residues and the fluorophore center of Rhodamine Red was estimated with an approximate maximal length of 1 nm.

3. Results

3.1. A fluorescent dye partially fills the green gap in LHCII absorption and excitation

The fluorescent dye Rhodamine Red was bound to LHCII to fill the green gap in the LHCII absorption and excitation spectra. The sulfhydryl-reactive maleimide derivative of the dye was coupled either to the single cysteine residue in the recombinant LHCII derivative S3Ch or to the three cysteines in 3xCh. S3Ch carries a single cysteine in position 3, near the N-terminus. 3xCh contains two additional cysteines, one in the lumenal loop at position 106, and one in the stromal loop at position 160. The bacterially expressed proteins were labeled, subsequently reconstituted with pigments to form trimeric LHCII, and purified. As controls, the unlabeled proteins were reconstituted and purified.

The absorption spectra of both dye-labeled LHCII derivatives, S3Ch and 3xCh (Fig. 1A and B, respectively), show a distinct absorption band at 573 nm, near the absorption maximum of Rhodamine Red in detergent solution (0.1% β-dodecylmaltoside) at 566 nm, in the middle of the green gap. The difference spectra between the dye-labeled and non-labeled complexes resemble the Rhodamine Red absorption spectra. As expected, in 3xCh with its 3 potential binding anchors, the contribution of the dye to the overall absorption is higher than in S3Ch with its single cysteine. Assuming that the extinction coefficient of protein-bound Rhodamine Red is the same as the one in methanol solution, we calculated dye–protein ratios of 0.63 and 2.58 in S3Ch and 3xCh, respectively.

Covalent binding of Rhodamine Red to LHCII was verified by denaturing the complex using the strong detergent sodium dodecyl sulfate and analyzing the protein component by denaturing polyacrylamide gel electrophoresis. The protein bands of the labeled but not of the unlabeled complexes exhibited fluorescence (not shown). The specificity of Rhodamine Red maleimide attachment to cysteines

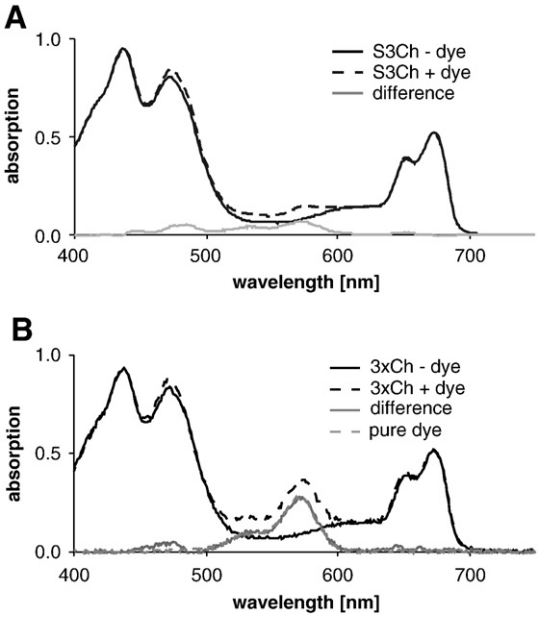

Fig. 1. Labeling with Rhodamine Red partially fills the green gap in LHCII absorption. Absorption spectra of unlabeled (solid black line) and labeled LHCII (dashed black line) were normalized at 670 nm. Difference spectra (solid grey line) were calculated, pure Rhodamine Red (dashed grey line in B) is normalized to the difference spectra. (A) S3Ch, (B) 3xCh.

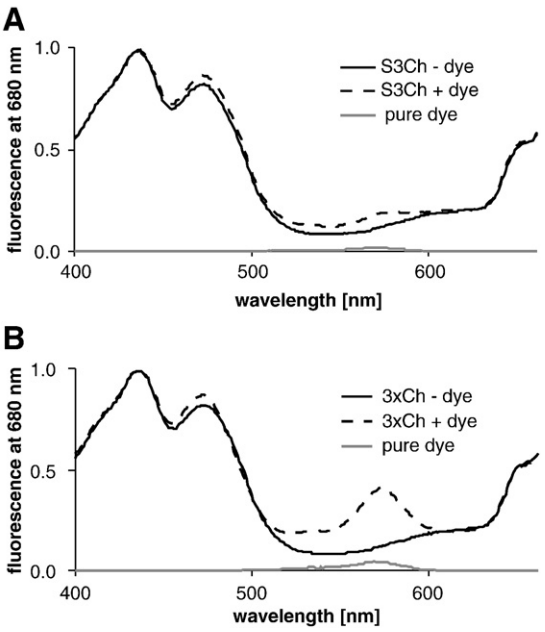

Fig. 2. Labeling with Rhodamine Red fills the green gap in the LHCII excitation spectrum. Excitation spectra ($\lambda_{em} = 680$ nm) of LHCII trimers with (dashed black line) and without (solid black line) Rhodamine Red. Spectra were normalized at 437 nm; absorption of LHCII never exceeded 0.05. Spectra of pure dye (grey line) were measured in the same buffer and had the same concentration as calculated for the labeled samples by absorption difference spectra. (A) S3Ch, (B) 3xCh.

was confirmed by a control experiment using the LHCII derivative C79S containing no cysteine; the apparent dye/protein ratio obtained with this protein was less than 10% (not shown).

Contribution of Rhodamine Red to LHCII absorption does not necessarily imply that the dye is able to add to the light-harvesting function of the complex. Therefore, we measured excitation spectra with the detection wavelength set to 680 nm, the emission wavelength of Chl *a*. As seen in Fig. 2, the contribution of the dye to the excitation spectra is about the same as that to the absorption spectra. Only a small part of that is due to emission of Rhodamine Red itself at 680 nm, since a control measurement with an aqueous solution of Rhodamine Red at the concentration as in the corresponding labeled LHCII samples yields only a small excitation signal. Thus, Fig. 2 demonstrates that Rhodamine Red excitation stimulates Chl *a* emission at 680 nm. Consequently, the dye is able to transfer its excitation energy efficiently to Chl *a* and thus contributes to the absorption cross section of the light-harvesting unit.

3.2. Donor quenching is nearly 100%

If a fluorophor transfers its energy to an acceptor, its emission (donor emission) is quenched. This effect can be used to quantitate fluorescence resonance energy transfer (FRET). Fig. 3 shows the fluorescence emission spectra of the donor Rhodamine Red in aqueous solution (solid grey lines). Its concentration is the same as the dye concentration in a 37 nM solution of labeled S3Ch and 3xCh (Fig. 3A and B, respectively). The excitation wavelength is 550 nm, near the absorption maximum of the dye. Unlabeled LHCII excited at this wavelength, emits some fluorescence (spectra indicated by the dark grey area in Fig. 3A, B) since even in the green gap LHCII shows some light absorption. When the labeled LHCII complexes are excited at the same wavelength (spectra indicated by the dashed areas), the donor emission at 573 nm disappears virtually completely, whereas Chl *a* emission at 680 nm is markedly enhanced. In the case of excitation energy transfer, the percentage of donor fluorescence quenched in the presence of the acceptor is a direct measure of the energy transfer

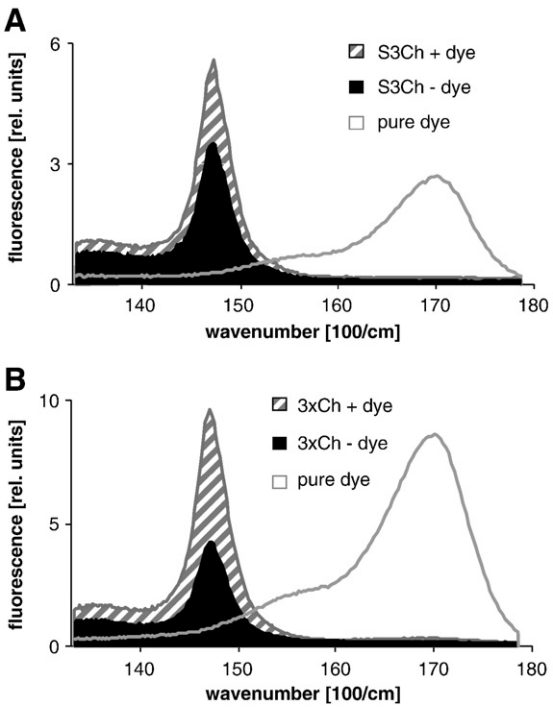

Fig. 3. Energy transfer measurements. Emission spectra ($\lambda_{ex} = 550$ nm) of LHCII with (dashed area) and without (solid area) Rhodamine Red. Concentration of LHCII was always 37 ± 3 nM. Spectra of pure dye (grey line) were measured in the same buffer and had the same concentration as calculated for the labeled samples by absorption difference spectra. (A) S3Ch, (B) 3xCh.

efficiency, provided the fluorescence quantum yield of the donor is not altered by some other effect, for instance a change in the donor dye's environment. In fact, upon labeling CP29, another Chl *a*/b-containing light-harvesting protein, with the fluorescent dye TAMRA, the dye's fluorescence was partially quenched even in the absence of pigments, presumably due to the protein environment [35]. We compared the fluorescence emission of Rhodamine attached to the LHCII apoprotein with that of the dye bound to an 8-amino acid fragment resulting from protease treatment of the labeled protein. We observed no difference in Rhodamine fluorescence emission between these two environments (not shown), which makes it unlikely that the environment in the pigmented protein has a large impact on the fluorescence quantum yield. Consequently, the energy transfer efficiency between Rhodamine and Chl, calculated from donor quenching, amounts to 98% for S3Ch and 99% for 3xCh.

If in fact this quenching is due to energy transfer, then the sensitized acceptor emission, i.e. the extra acceptor emission seen only in the presence of the donor, should correspond to the quenched donor emission. If both donor and acceptor have the same fluorescence quantum yields φ_F then the disappearing area under the quenched donor spectrum (A_Q) should be same as the added area under the sensitized acceptor spectrum (A_S), with both spectra plotted against wavenumbers rather than wavelengths. If the φ_F values are not the same, then

$$A_S = [\varphi_F(\text{acceptor}) / \varphi_F(\text{donor})]^* A_Q. \quad (3)$$

With φ_F (Rhodamine Red) = 0.5 and φ_F (LHCII) = 0.2 (see Materials and methods), A_S should be $0.4^* A_Q$. The ratios A_S / A_Q taken from Fig. 3, 0.42 for S3Ch and 0.34 for 3xCh, are in good agreement with the expected values. We conclude that in Rhodamine Red-labeled S3Ch and 3xCh, the dyes transfer their excitation energy to the Chls at an efficiency of 98% and 99%, respectively.

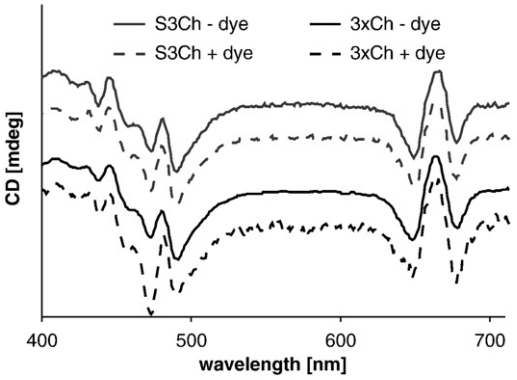

Fig. 4. Protein folding and trimerization is not affected by labeling. CD spectra of labeled (dashed line) and unlabeled (solid line) LHCII. Spectra were normalized at 490 nm; grey: S3Ch, black: 3xCh.

3.3. Labeling does not significantly affect the fluorescence quantum yield, structure, pigment binding, and stability of LHCII

If dye-labeled LHCII is to be used as an improved light harvester, then it is essential that labeling does not reduce the fluorescence quantum yield of the Chls in LHCII or the structure or stability of the complex. To compare the Chl fluorescence quantum yields of labeled and non-labeled LHCII, the emission spectra of complex solutions were measured with the excitation wavelength set to 470 nm where Chl *b* absorbs efficiently whereas Chl *a* and Rhodamine Red absorb to a much lesser extent. In properly assembled LHCII, Chl *b* transfers its excitation energy entirely to Chl *a* so that upon excitation of Chl *b*, emission occurs only from Chl *a* at 680 nm but not from Chl *b* at 660 nm. The integrated intensities of labeled and non-labeled LHCII, all at a concentration of 37 nM, were the same within $\pm 4\%$ and no shoulder at 660 nm was seen (not shown). Therefore, the presence of the dye significantly affects neither the quantum yields of Chls in LHCII nor the ability of Chl *b* to transfer its excitation energy to Chl *a*.

Proper folding and assembly with pigments of the labeled LHCII apoprotein to yield the authentic LHCII structure were further tested by comparing the CD spectra in the visible domain of labeled complexes with those of the non-labeled ones (Fig. 4). The CD signals of LHCII are due to a multitude of pigment–pigment interactions [36] and can therefore be used as a fingerprint of the correctly assembled pigment–protein complex. The CD spectra of the unlabeled LHCII versions were very similar to that of wild type LHCII [27]. Most features in the CD signals of labeled LHCII were also identical except the negative band at 470 nm. This band was somewhat more prominent in labeled S3Ch and significantly enhanced in labeled 3xCh.

Pigment binding of the samples was tested by butanol extraction and HPLC analysis. Determined stoichiometries were calculated on a 2 lutein basis as proper occupation of the lutein binding sites is essential for a stable LHCII [37]. All pigment–protein complexes in this study exhibited similar stoichiometries (Table 1). Total Chl levels for all samples were between 13.6 and 14.2 with a Chl *a*/*b* ratio of 1.19 (unlabeled S3Ch) to 1.31 (labeled 3xCh). Neoxanthin (Neo) as the only carotinoid bound besides lutein amounted to 0.79 ± 0.05 per 2 luteins.

Table 1
Pigment stoichiometries[a] as determined by HPLC.

Sample	Neo[b]	Chl *b*[b]	Chl *a*[b]
S3Ch − dye	0.84	6.22	7.42
S3Ch + dye	0.82	5.99	7.77
3xCh − dye	0.77	6.27	7.89
3xCh + dye	0.72	6.01	7.86

[a] Mean values of 2–3 independent measurements.
[b] mol pigments per 2 mol lutein.

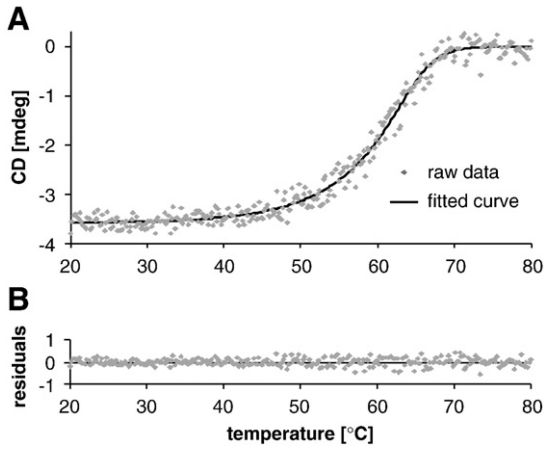

Fig. 5. Labeling has no influence on thermal stability. (A) $CD_{492\ nm}$ trace during a temperature gradient of labeled 3xCh together with a fitted curve (solid line), (B) residuals of fitted curve.

These data match with pigment stoichiometries of other recombinant LHCII [38] and no significant differences were detected between the two LHCII mutants or between labeled and unlabeled complexes.

The thermal stability of labeled and unlabeled LHCII versions was tested by measuring its temperature-dependent dissociation, monitored by the negative CD signal at 492 nm. This CD band disappears when monomeric LHCII falls apart [39]. Fig. 5 shows the "melting curve" of labeled 3xCh; data from S3Ch and both non-labeled LHCII versions were very similar. Below 25 °C and above 75 °C the CD signal at 492 nm showed no temperature dependence. The inflection points of the fitted curves were taken as the "melting temperature". This temperature was within the range of (60.8 ± 0.6)°C for all four samples, indicating that labeling LHCII with up to three Rhodamine Red dyes did not significantly affect complex stability (Table 2).

4. Discussion

4.1. Energy transfer mechanism

The Rhodamine Red-labeled LHCII derivatives presented here exhibit nearly 100% donor (Rhodamine) quenching and an approximately equivalent amount of sensitized acceptor (Chl) fluorescence. Taken together, this strongly suggests energy transfer between the chromophores. The closest distance between the Cα atom of the labeling positions and a Chl is 9.3 Å (S160 to Chl 11, [12]), which is beyond van-der-Waals distances. Therefore, energy transfer presumably follows the Förster FRET mechanism [40]. The estimated distances between Rhodamine dyes in positions 106 and 160 and the nearest Chl are 2.4 nm to Chl 14, and 1.9 nm to Chl 11, respectively (position 3 is not resolved in the crystal structure). These distances are much shorter than the critical Förster distances R_0 (4.7 nm and 4.6 nm for energy transfer to Chl *a* and Chl *b*, respectively). Therefore, Förster energy transfer efficiencies between 97.5 and 99.5% are expected. This is still a lower limit since only the nearest Chl to each dye is taken into account. Actually, each donor dye can transfer its excitation energy to a number of acceptor Chls which will make energy transfer even more efficient.

The sensitized acceptor emission matches donor quenching in reasonable approximation. The slight difference is most likely due to an imprecision in the parameters to be taken into account.

4.2. Impact of labeling on LHCII folding and stability

It is remarkable that LHCII can accommodate three bound chromophores without its structure or stability being significantly affected. This has been shown by the energy transfer between Chls and the thermal disintegration staying unchanged upon labeling. The CD spectrum of the triple-labeled LHCII, too, shows all the peaks characteristic for LHCII trimers [27]. The only difference between labeled and unlabeled complexes is the enhanced negative band at 473 nm. This signal increase has been observed upon the loss of bound Neo [36,41–43]. However the pigment stoichiometries measured show no pigment loss, including Neo, upon Rhodamine Red labeling. Minor structural changes have been suggested to be able to alter this CD signal [36]. Such minor structure rearrangements may also be responsible for the slight changes observed in the absorption around 470 nm of labeled samples. Similarly, amino acid exchanges in the luminal loop of LHCII have been shown to affect the negative CD band at 473 nm as well as the absorption around 470 nm without significantly affecting the Neo stoichiometry, possibly by slightly altering the arrangement or environment of Neo [44]. Rhodamine Red in position 106 would be the most likely candidate for exhibiting a similar effect on Neo.

4.3. Labeling efficiencies

In this study we used LHCII mutants with one or three cysteines exhibiting dye–protein ratios of 0.63 and 2.58 respectively. Since all labeling positions are in loop or terminal regions, they should be readily accessible to the reactive dyes and, therefore, be labeled at a high efficiency. One possible reason for incomplete labeling is electrostatic repulsion between dye and protein. Rhodamine Red has a positive charge delocalized over the conjugated system, and especially the cysteine in position 3 has positive charges in close proximity, namely Arg1 and Lys2. In fact, labeling of this position in S3Ch is only 63% efficient. If in the triple-labeled protein, too, only about 2/3 of the cysteines in position 3 bind a dye, then the total dye: protein ratio of 2.58 implies that the remaining two positions are virtually completely labeled.

If the triple-labeled protein 3xCh had the full 3:1 ratio of dye:protein, then the expected contribution of Rhodamine Red to the light-harvesting efficiency of LHCII would be some 15% higher than the one obtained in this work. A larger increase in efficiency would be expected if additional donor dyes would be introduced into LHCII. The additional labeling sites would have to be in exposed regions of the LHCII protein so as to minimize the risk of the dye interfering with the LHCII pigment–protein structure. Moreover, the labeling sites should have some distance to one another to avoid close interaction between the dyes that may lead to dissipation of excitation energy. We have added a fourth cysteine anchor near the C-terminus of the LHCII protein. The quadruple-labeled protein was fully active in its reconstitution with pigments; however, trimerization of the complex was significantly impaired. Since structural changes in the C-terminal domain have been known to interfere with trimer formation of LHCII [45], this disturbance may be attributed to the fourth label itself and not to the fact that as many as four fluorophores are bound to the complex. Therefore, the possibility is still open to increase the light-harvesting efficiency of LHCII by more than three dyes without any negative impact on its structure or function.

5. Conclusion

In this study we present a hybrid structure of organic dye molecules and a biological protein. Up to three Rhodamine Red molecules covalently bound to each LHCII apoprotein contribute to LHCII absorption in its green gap without significantly affecting the

Table 2
Inflection points of "melting curves".

Sample	Inflection point
S3Ch − dye	61.3 °C
S3Ch + dye	61.4 °C
3xCh − dye	60.3 °C
3xCh + dye	60.5 °C

structure or stability of trimeric LHCII. This construct may therefore be useful for biomimetic light-harvesting in photovoltaic and other applications. LHCII is not photostable under long-term exposure to strong light and the cost of its production in the laboratory is rather high; this limits its usefulness on a technical scale. On the other hand, its capacity of self-organization and, thus, self-repair may make up for some of these obstacles.

Acknowledgement

We thank the Volkswagen Foundation (Germany, I/82 072-074) for financial support.

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